Interleukin-21 promotes the development of ulcerative colitis and regulates the proliferation and secretion of follicular T helper cells in the colitides microenvironment

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Abstract. Patients with ulcerative colitis (UC) are at increased risk of developing colitis-associated colon cancer. Previous studies have indicated that interleukin (IL)-21, which is predominantly secreted by follicular T helper (Tfh) cells, is overproduced in inflammatory bowel diseases. In order to investigate the role of IL-21 in UC and the association between IL-21 and Tfh cells, the number of Tfh cells and the level of IL-21 were investigated in colonic tissues from UC patients and wild-type (WT) mice, which were induced by dextran sulphate sodium (DSS). High Tfh cell counts and levels of IL-21 were observed in UC patients and WT mice with DSS-induced colitis. Subsequent comparison of the mucosal damage and expression of Tfh-associated cytokines in the WT mice and IL-21 knockout (IL-21KO) mice following DSS administration, revealed that IL-21KO mice were largely protected against colitis and exhibited reduced infiltration of Tfh cells, as well as decreased production of Tfh-associated cytokines. The present study also found that IL-21 was necessary for the proliferation and secretion of Tfh cells *in vitro*. In addition, neutralization of IL-21 in DSS-administered WT mice using anti-IL-21 reduced the number of Tfh cells and the level of mucosal damage. Administration of a neutralizing IL-21 antibody decreased the colonic infiltration of Tfh cells and reduced damage to the mucosa. These results indicated that Tfh cells are important in UC and that its effector molecule, IL-21, is not only a critical regulator of inflammation, but also regulates the proliferation and response of Tfh cells in the colitis microenvironment.

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Introduction

Chronic inflammation is a major driving force in the initiation and progression of various types of tumor in numerous tissues (1). Ulcerative colitis (UC) is one of the major forms of chronic inflammatory bowel diseases in humans. Patients with UC have an increased risk of developing colitis-associated colon cancer (CAC) and the level of risk is associated with the extent and duration of the disease and the severity of microscopic inflammation (2). There are different opinions regarding the mechanisms underlying UC (3-5). Immune cells, including T helper (Th)1, Th17 and follicular T helper (Tfh) cells, infiltrate the nidus producing cytokines, which is considered to be important in the progression of inflammation.

Tfh cells, which are located in germinal centers (GC), have emerged as a separate subset of cluster of differentiation 4 $(CD4)^+$ T helper cells that express high levels of inducible costimulator (ICOS), C-X-C chemokine receptor type 5 (CXCR5), CD40 ligand (CD40L), programmed cell death-1 (PD-1) and the important transcription factor B-cell lymphoma 6 (Bcl-6) (6,7). Interleukin (IL)-21 is a pleiotropic cytokine synthesized by Tfh cells and is also expressed by other $CD4^+$ T cells, including Th1, Th17 and activated natural killer T cells (8-10). IL-21 modulates innate and adaptive immune responses (11). Furthermore, IL-21 is crucial in the proliferation of T cells, B cells and natural killer cells, and affects regulatory T cells (12). IL-21 performs functions through a heterodimeric receptor, IL-21 receptor (IL-21R), which is highly expressed by $CD4^+$ T cells (13,14). Studies have indicated that IL-21 controls the functional activity of epithelial cells and fibroblasts in the gut, thus it is an important mediator in the crosstalk between immune and nonimmune cells (15,16). The present study examined whether Tfh cells and IL-21 are necessary for the development of chemically-induced colitides and examined their interconnections in UC.

Materials and methods

Patients. Colonic tissue sections were obtained from patients comprising 20 cases of UC and 15 healthy controls, the latter

suffering from non-UC inflammatory diseases, including colonic ileus and vascular malformation. The diagnosis of UC was determined according to established guidelines based on endoscopic and histopathological examination. Each sample was divided into two sections. One section was used for histopathological analysis, in which the sample was deparaffinized, dehydrated through xylene and ethanol and incubated with a rabbit anti-human polyclonal IL-21 antibody (R&D Systems, Minneapolis, MN, USA) for 2 h at room temperature. It was then counterstained with hematoxylin (Sangon Biotech, Shanghai, China). The other section was frozen in liquid nitrogen and stored at -80˚C until Tfh cell and IL-21 RNA analysis. All the samples were collected at the Department of General Surgery, The First Affiliated Hospital of Soochow University (Suzhou, China), between February 2012 and March 2013. The human studies were approved by the ethics committee of the First Affiliated Hospital of Soochow University and each patient provided written informed consent.

Animals. Female wild-type (WT) and IL-21 knockout (IL-21KO) mice, 6-8-weeks of age, with the same genetic background (C57BL/6J), were purchased from the Chinese Academy of Sciences (Shanghai, China). The mice were bred and maintained under specific pathogen-free conditions in the animal facility at the Experimental Department of The First Affiliated Hospital of Soochow University. The IL-21KO mice were viable and did not exhibit any phenotype. All animal experiments were approved by the local (the Regulation of Animal Experiments, 1988.10.31) and national (Measures of Jiangsu Province on Administration of Affairs Concerning Experimental Animals, 200.10.01) institutional guidelines.

Model of UC. On the basis of the preliminary experiments (unpublished data), the WT and IL-21KO mice were administered 3% dextran sulphate sodium (DSS) in drinking water for 14 days. The control experiment animals received water only. The result of the preliminary study demonstrated that 100% of the WT mice developed colitis at day 14.

Histological examination. Colonic tissues of the mice treated with DSS were removed and fixed in formalin solution overnight at 4˚C, embedded in paraffin, processed, sectioned at a thickness of 5 μm and stained with hematoxylin & eosin (H&E). Colitis was graded at day 4 using a scale, which was originally developed for grading inflammation in UC patients (17). The samples were graded as follows: Grade 1, normal mucosa; grade 2, mild inflammation (gland enlargement, many intraepithelial granulocytes, and influx of cells and/or eosinophils into the stroma); grade 3, intermediate inflammation (gradual reduction in goblet cells, tubular parallelism, mucin secretion and aggregation of inflammatory cells in the stroma); grade 4, severe inflammation (obvious atrophy of glands and mucosa, abundant proliferation of inflammatory cells and follicle formation in deeper cell layers, crypt abscesses and pus on the surface); and grade 5, fulminated inflammation (ulceration with pus, gland and mucosal atrophy, crypt abscesses, and inflammation of the stroma and deep follicles). The average score of the WT mice group and IL-21KO mice group in the examination was then calculated and compared.

RNA preparation and reverse transcription quantitative polymerase chain reaction (RT-qPCR). Total RNA was extracted from the tissues using an miRNeasy minikit, according to the manufacturer's instructions (Invitrogen Life Technologies, Carlsbad, CA, USA). A sample of total RNA (1 μg) was used to produce first-strand cDNA with a random primer (Random Hexamers Primer) in a final reaction volume of 40 μl. The RNA templates were treated with DNase to avoid genomic DNA contamination. The cDNA/sample (1 μl) was then amplified by qPCR in an Applied Biosystems 7300 detection system (Applied Biosystems, Foster City, CA, USA). Combined murine primers were purchased from Shanghai Generay Biotech Co., Ltd. (Shanghai, China). The following primers were used: IL-21, forward 5'-CGCCTCCTGATTAGACTTCG-3' and reverse 5'-TGGGTGTCCTTTTCTCATACG-3'; ICOS, forward 5'-TGACCCACCTCCTTTTCAAG-3' and reverse 5'-TTAGGGTCATGCACACTGGA-3'; CXCR5, forward 5'-CTTCGCCAAAGTCAGCCAAG-3' and reverse 5'-TGGTAGAGGAATCGGGAGGT-3'; PD-1, forward 5'-AAGCTTATGTGGGTCCGGC-3' and reverse 5'-AAGCTTATGTGGGTCCGGC-3'; Bcl-6, forward 5'-CAGATTTGTACAGGTGGCCCA-3' and reverse 5'-AGAGTCTGAAGGTGCCGGAA-3' and GAPDH, forward 5'-AGAACATCATCCCTGCATCC-3' and reverse 5'-AGCCGTATTCATTGTCATACC-3'. qPCR reactions were performed according to the manufacturer's instructions of the iQ SYBR Green Supermix (Takara Bio, Inc., Shiga, Japan). Data was normalized with the levels of GAPDH in the samples. Human IL-21RNA was evaluated using a TaqMan assay (Applied Biosystems).

Protein extraction and ELISA analysis. IL-21 in the colonic tissues of mice was measured using ELISA, according to the manufacturer's instructions (Sangon Biotech, Shanghai, China). Optical densities were measured at 450 nm using a Multiskan MK3 (Thermo Fisher Scientific, Waltham, MA, USA; purchased from Vedeng, Nanjing, China).

Lamina propria mononuclear cell (LPMC) isolation. LPMCs were isolated, as follows: Colonic tissues from human and mice were cut longitudinally and washed with Hank's balanced salt solution (HBSS; Sangon Biotech) to remove feces and debris. The colon sections were then finely minced and incubated in HBSS containing 10% fetal bovine serum (FBS; Hangzhou Evergreen Biological Engineering Materials Co., Ltd., Hangzhou, China), 0.145 mg/ml dithiothreitol, 5 mM EDTA, 1% penicillin/streptomycin (P/S) and 1 M Hepes (all from the laboratory of The First Affiliated Hospital of Soochow University) at 37˚C for 15 min for two cycles. The EDTA was then removed by washing three times in HBSS and the tissue was digested in RPMI-1640 (Hangzhou Evergreen Biological Engineering Materials Co., Ltd.) containing 0.4 mg/ml collagenase D (Sangon Biotech) and 0.01 mg/ml DNase I (Sangon Biotech) for 60 min at 37˚C on an agitating platform. Following collagenase digestion, the medium containing the mononuclear cells was collected and centrifuged at 500 x g for 10 min and the resulting cells were resuspended in RPMI-1640 supplemented with 10% FBS and 1% P/S. The LPMCs were incubated for 50 min with PE-conjugated CD4 (eBioscience, San Diego,

CA, USA), FITC-conjugated CXCR5 (R&D Systems) and PerCP/Cy5.5-conjugated ICOS (BioLegend, San Diego, CA, USA) at 4˚C and washed twice with staining buffer (1X PBS, 2 mmol EDTA and 0.5% BSA). The cells were then resuspended in 400 ml PBS and assayed using flow cytometry (Beckman Coulter, Brea, CA, USA). The percentages of $CXCR5^+$ $ICOS^+$ cells in $CD4^+$ lymphocytes were determined. The cells were then analyzed using the supporting flow cytometry software (Cytomics FC 500, Beckman Coulter, Brea, CA, USA).

Lamina propria T lymphocyte culture and IL-21 analysis. $CD3^+$ lamina propria T lymphocytes were purified from the LPMC using magnetically labeled CD3 antibodies (R&D Systems), resuspended in RPMI-1640 supplemented with 10% FBS (Hangzhou Evergreen Biological Engineering Materials Co., Ltd.) and cultured with anti-CD3 beads (Miltenyi Biotec, Bergisch Gladbach, Germany). They were then divided into three equal portions and added to 10 μg/ml anti-IL-21 (eBioscience), 10 μg/ml control IgG (eBioscience) and 10 μg/ml IL-21 (eBioscience), respectively. Following culture for 72 h, these were used for flow cytometric analysis and RNA extraction.

Statistical analysis. The data were analyzed using SPSS 17.0 statistical software (SPSS, Inc., Chicago, IL, USA). Differences among the groups were compared using Student's t-test, analysis of variance and Wilcoxon tests. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Overexpression of Tfh cells and IL-21 in human and mouse UC. The percentage of $ICOS^+$ $CXCR5^+$ cells among the $CD4^+$ T lymphocytes were examined and were considered to represent Tfh cells (17). The results of the present study revealed a significant upregulation of Tfh cells among the $CD4^+$ T lymphocytes in the UC patients compared with those in the controls (Fig. 1A and B). Immunohistochemistry was then used to assess the expression of IL-21 in the mucosa of patients with UC and in the controls. The results confirmed the increased expression of IL-21 in UC (Fig. 1C). In addition, more pronounced RNA expression of IL-21 was detected in patients with UC compared with the controls (Fig. 1D).

IL-21-deficient mice are resistant to DSS colitis and produce fewer Tfh cells. WT and IL-21KO mice were treated with DSS and the degree of inflammation was assessed by histological examination at day 14. The WT mice demonstrated marked gland and mucosal atrophy, evident crypt abscesses with pus on the surface, a considerable influx of acute inflammatory cells and follicle formation in deeper cell layers. By contrast, IL-21-deficient mice demonstrated mild infiltration of inflammatory cells in the mucosa, with a reduction in goblet cells, minimal loss of crypts and lymphoid aggregates (Fig. 2A). Furthermore, the average inflammatory grade score of the IL-21KO mice was lower than the WT mice (Fig. 2C). In order to examine whether the level of IL-21 was associated with Tfh cell differentiation and proliferation, the percentage of Tfh cells was compared between the WT and IL-21KO mice. It was found that the percentage of Tfh cells in the WT mice was significantly higher than in the IL-21KO mice regardless of DSS treatment and Tfh cells were increased in the WT mice, however, not in the IL-21KO mice, following DSS treatment (Fig. 2B).

Subsequently, the expression of Tfh cells and IL-21 in mice with DSS-induced colitis was analyzed. WT mice were provided with DSS-supplemented water (3%) and then sacrificed at day 14. ELISA and qPCR analysis of IL-21 in the colonic extracts obtained from the DSS-treated and control mice revealed that the mRNA and protein levels of IL-21 were upregulated in colitis (Fig. 2D and E). Flow cytometric analysis of the mononuclear cells isolated from the DSS-treated and control mice also demonstrated that Tfh cells were increased in DSS-induced colitis (Fig. 2B).

IL-21-deficient mice are unable to upregulate Tfh-associated molecules following DSS treatment. To examine whether IL-21 controls Tfh cell response in UC, RNA was extracted from colonic specimens of WT and IL-21KO mice and the content of various mediators of Tfh cells was analyzed. Initially, the expression of PD-1, ICOS, CXCR5 and CD40L was analyzed. A significant increase in ICOS, CXCR5 and CD40L RNA was observed in the colonic tissues of mice that received DSS, independently of the presence of IL-21 (Fig. 3A, C and D), whereas PD-1 transcripts did not differ between the WT and IL-21KO mice (Fig. 3B). By contrast, following DSS treatment, the WT mice, but not the IL-21-deficient mice, demonstrated a significant increase in ICOS and CD40L (Fig. 3C and D), indicating that IL-21 was necessary for the induction of ICOS and CD40L during colitis. The expression of Bcl-6, a key transcription factor of Tfh cells, was then examined. Following DSS administration, the quantity of bcl-6 transcripts increased significantly in the WT, but not in the IL-21-deficient mice (Fig. 3E).

IL-21 is necessary for Tfh cell proliferation and secretion in vivo. The above results indicated that IL-21 was necessary for enhancing the proliferation of Tfh cells and the expression of Tfh-associated molecules. In order to confirm this, the $CD4^+$ T cells of WT mice were divided into three groups, and cultured and stimulated with anti-IL-21, control IgG or IL-21. Subsequently, the percentage of Tfh cells and its associated molecules were analyzed after 10 days. Flow cytometric analysis confirmed that the percentage of Tfh cells in the anti-IL-21 group was less than in the control IgG group and was highest in the IL-21 group (Fig. 4A). The possibility that IL-21 enhanced the secretion of Tfh cells was then examined. RNA was extracted from each group and analyzed by qPCR. The results demonstrated that the expression of ICOS and CD40L was lowest in the anti-IL-21 group and highest in the IL-21 group (Fig. 4B and C). The same results were observed for the key transcription factor, Bcl-6 (Fig. 4D).

Inhibition of endogenous IL-21 ameliorates DSS-induced colitis and reduces Tfh cells in WT mice. The aforementioned findings suggested that elevated levels of IL-21 in WT mice affected the chronic phase of DSS-induced colitis and enhanced the percentage of Tfh cells in the $CD4^+$ T lymphocytes. To assess this hypothesis, certain DSS-treated WT

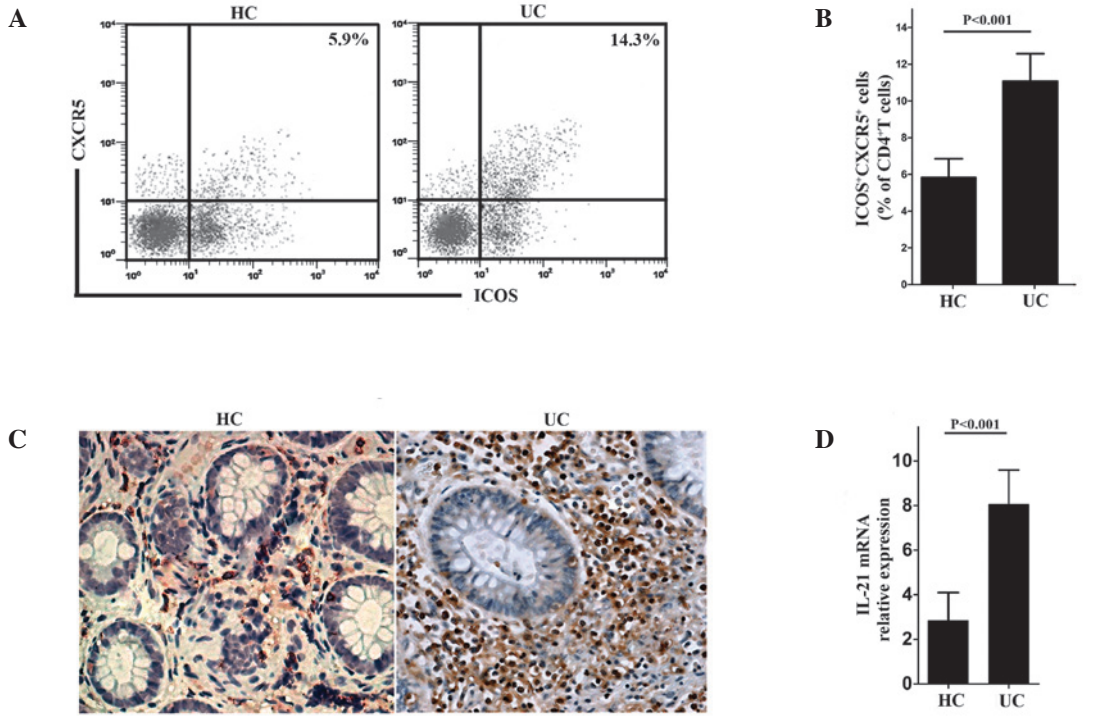

Figure 1. Tfh cells and IL-21 are overexpressed in human UC. (A) Percentage of ICOS$^+$ CXCR5$^+$ cells in the CD4$^+$ T lymphocytes of one patient and one HC. (B) Percentage of ICOS$^+$ CXCR5$^+$ cells in the CD4$^+$ T lymphocytes of 20 UC patients and 15 HCs. (C) IL-21 immunostaining in colonic tissues from one HC and one patient with UC. Magnification, x100. (D) RNA transcripts for IL-21 in fresh colonic specimens obtained from HC and UC patients and quantified by quantitative polymerase chain reaction. Tfh, follicular T helper; IL, interleukin; UC, ulcerative colitis; HC, healthy control; ICOS, inducible costimulator; CXCR5, C-X-C chemokine receptor type 5; CD4, cluster of differentiation 4.

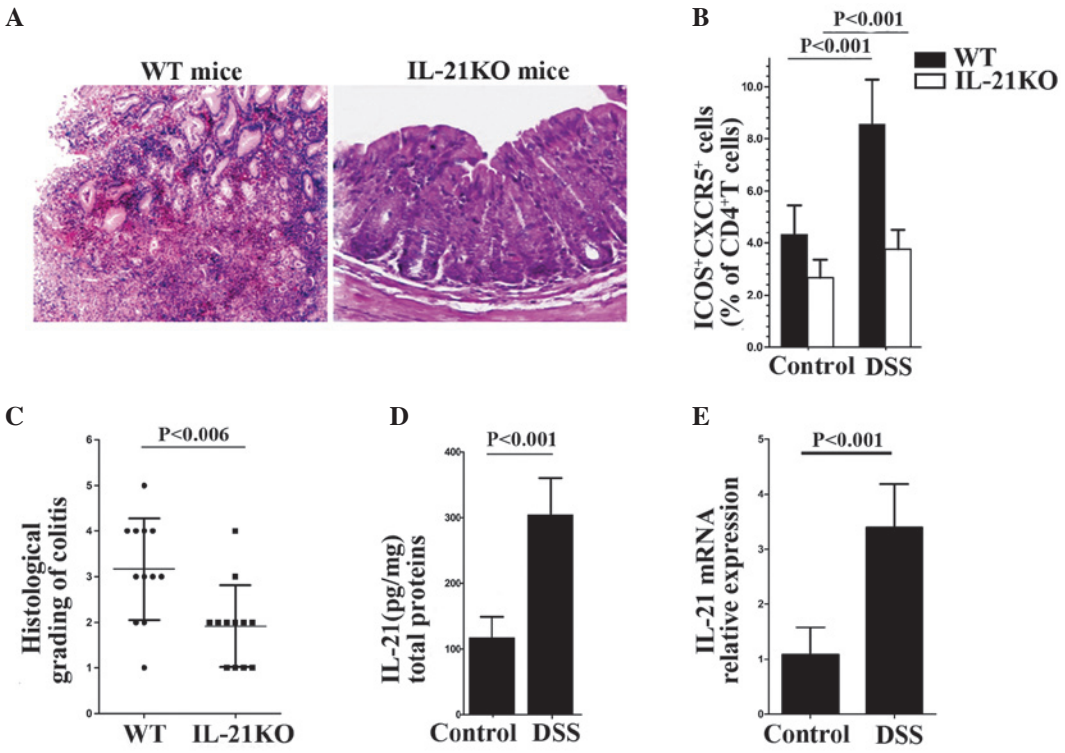

Figure 2. Induction of DSS-colitis in WT mice is associated with enhanced Tfh cell proliferation and IL-21 synthesis. IL-21-deficient mice were resistant to DSS colitis and produced fewer Tfh cells. (A) Representative hematoxylin & eosin-stained sections of the colon obtained from WT and IL-21KO mice. Induction of colitis in IL-21-deficient mice demonstrated mild mucosal infiltration of inflammatory cells and a minimal reduction in goblet cells. Magnification, x20. (B) Percentage of ICOS$^+$ CXCR5$^+$ cells in the CD4$^+$ T lymphocytes of mice colonic tissues. (C) Inflammatory score of 12 WT mice and 12 IL-21KO mice following DSS treatment. (D) IL-21 protein was analyzed by ELISA in the colonic extracts of WT mice treated with or without DSS. Data are expressed as picogram/milligram total protein. In each experiment, six mice per group were used. (E) The mRNA expression of IL-21 in the colonic extracts of WT mice with or without DSS treatment. IL-21KO, interleukin-21 knockout; WT, wild-type; DSS, dextran sulphate sodium; IL-21, interleukin-21; Tfh, follicular T helper; ICOS, inducible costimulator; CXCR5, C-X-C chemokine receptor type 5; CD4, cluster of differentiation 4.

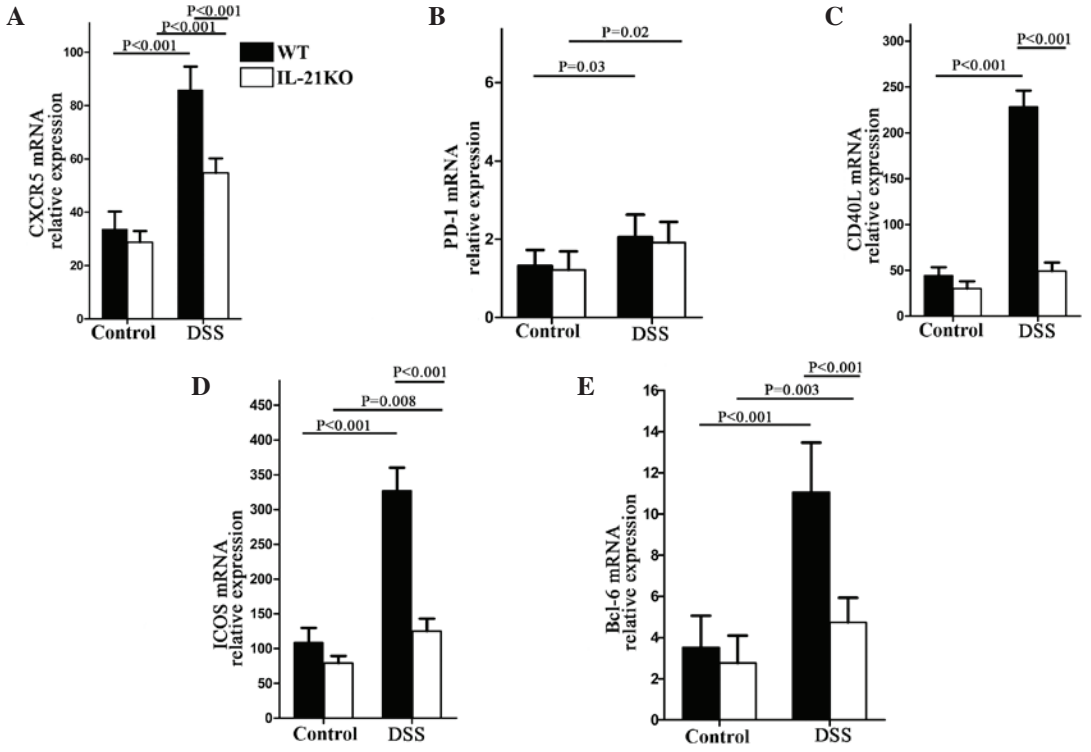

Figure 3. WT and IL-21KO mice were treated with or without oral DSS and sacrificed for the collection of colonic samples at day 14. The expression of the Tfh-associated markers, CXCR5, ICOS, CD40L, PD-1 and Bcl-6, were analyzed by quantitative polymerase chain reaction. In each experiment, six mice per group were used; (A) CXCR5, (B) PD-1, (C) CD40L, (D) ICOS and (E) Bcl-6. WT, wild-type; IL-21KO, interleukin-21 knockout; DSS, dextran sulphate sodium; Tfh, follicular T helper; CXCR5, C-X-C chemokine receptor type 5; ICOS, inducible costimulator; CD40L, CD40 ligand; PD-1, programmed cell death-1; Bcl-6, B-cell lymphoma 6.

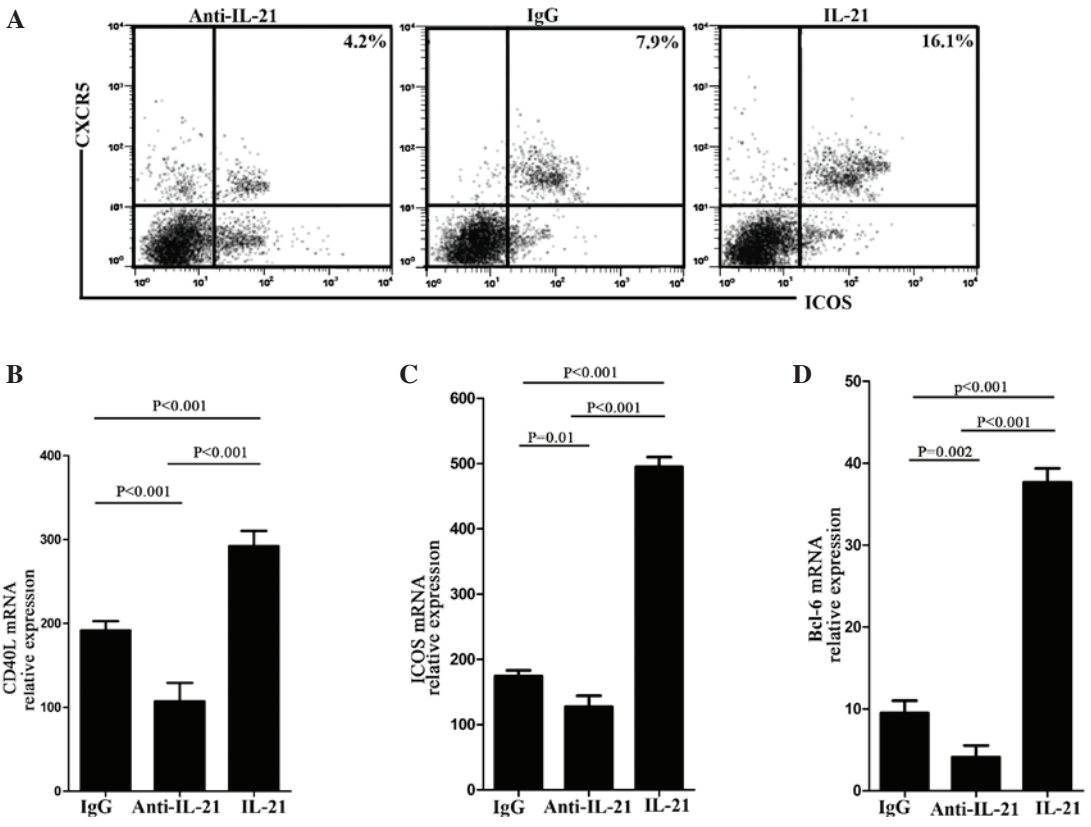

Figure 4. T cells from WT mice fail to promote the proliferation and secretion of Tfh cells. (A) Representative dot-plots showing ICOS$^+$ CXCR5$^+$ cells in a CD4$^+$ T cell culture system undergoing different treatments. CD4$^+$ T cells were obtained from the colonic tissues of WT mice without DSS treatment. (B-D) The expression of Tfh-associated markers, ICOS, CD40L and the transcription factor Bcl-6 was analyzed by quantitative polymerase chain reaction. In each experiment, six mice per group were used. WT, wild-type; Tfh, follicular T helper; DSS, dextran sulphate sodium; ICOS, inducible costimulator; CXCR5, C-X-C chemokine receptor type 5; CD4, cluster of differentiation 4; CD40L, CD40 ligand; Bcl-6, B-cell lymphoma 6.

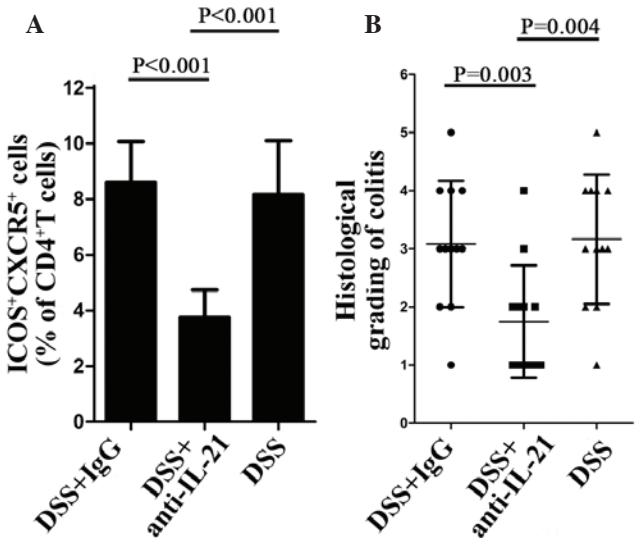

Figure 5. WT mice treated with IL-21 neutralizing antibody (anti-IL-21) are less susceptible to UC and unable to upregulate Tfh cells. (A) Percentage of ICOS$^+$ CXCR5$^+$ cells in CD4$^+$ T lymphocytes. CD4$^+$ T lymphocytes were isolated from WT mice following DSS + IgG, DSS + anti-IL or DSS treatment, respectively. (B) Inflammatory score of WT mice following DSS + IgG, DSS + anti-IL or DSS treatment, respectively. WT, wild-type; Tfh, follicular T helper; IL, interleukin; IgG, immunoglobulin G; DSS, dextran sulphate sodium; CD4, cluster of differentiation 4; ICOS, inducible costimulator; CXCR5, C-X-C chemokine receptor type 5.

mice randomly received either neutralizing IL-21 (anti-IL-21; 150 μg/mouse) or control IgG (150 μg/mouse), once per week intraperitoneally (eBioscience) until day 14. Flow cytometric analysis of the CD4$^+$ T cells isolated from the anti-IL-21-treated and control mice indicated that Tfh cells were decreased in the anti-IL-21-treated mice (Fig. 5A). Histological examination at day 14 demonstrated that inhibition of endogenous IL-21 significantly reduced the inflammatory score compared with the control mice (Fig. 5B).

Discussion

Tfh cells have attracted increasing attention in recent years and have been demonstrated to be involved in several immune diseases (18-20). As a separate subset of CD4$^+$ T helper cells, the relevant transcription factor for these cells is Bcl-6, which distinguishes them from Th1, Th2 and Th17 cells (21,22). These Tfh cells express high levels of CXCR5, which allows their chemotaxis and retention in the lymphoid follicle and promotes the formation of the GC. ICOS, CD40L and PD-1 are markers of Tfh cells (6,7), whereas IL-21 is the major cytokine secreted by Tfh cells and has a dual function in inflammation (23). IL-21 is able to trigger the inflammatory pathway and promote tissue damage in numerous organs. The pathogenic effect of IL-21 has been described in psoriasis (24), rheumatoid arthritis (25), Type I diabetes (26) and systemic lupus erythematosus (27). The present study examined whether Tfh cells and IL-21 are involved in the process of DSS-induced colitis and examined the association between them.

Initially, the present study demonstrated upregulation of IL-21 in the colonic tissues of patients with UC, suggesting that IL-21 may be important in UC. This investigation was extended through examination of UC in animals and the same conclusion was reached. The present study provided evidence that IL-21-deficient mice were largely protected against the development of colonic inflammation. These results were confirmed by further studies demonstrating that WT mice (treated with DSS), which were administered with neutralizing IL-21 antibody, developed milder inflammation compared with treatment with a control antibody.

Although, the importance of IL-21 in various antibacterial responses and immune-inflammatory diseases has been understood for several years, it is now recognized that the majority of IL-21 is secreted by a separate Th cell subset, termed Tfh cells (6,28). Previous studies have demonstrated that the important function of Tfh cells is to assist B cells in producing high-affinity antibodies through cognate Tfh-B cell interaction in the GC of B cell follicles and to adjust antibody type conversion, therefore they are involved in autoimmune diseases (20). Few studies have indicated the involvement of Tfh cells in inflammatory disease, however, Tfh-associated cytokines, including CXCR5, IL-21 and ICOS play a decisive role in the production of chemokines (22,29). Therefore, the present study aimed to examine the interconnections between IL-21 and Tfh cells.

Analysis of the mechanisms underlying the IL-21-mediated progression of UC revealed that the lack of IL-21 was paralleled by a marked reduction in the number of Tfh cells, suggesting that IL-21 was not only an effector molecule, but also a key regulator of Tfh cells. These results were confirmed by further evidence.

Initially, whether the level of Tfh cells in WT mice is higher than in IL-21KO mice following treatment with DSS was examined. The results demonstrated that the expression of CXCR5, ICOS, CD40L and Bcl-6 were increased in WT, but not in IL-21KO, mice.

Secondly, the lack of IL-21 during culture of CD4$^+$ T cells caused a significant inhibition of Tfh cell proliferation and downregulated the expression of cytokines. The opposite effect was observed when stimulated with IL-21.

Finally, in WT mice administered with a neutralizing IL-21 antibody, the production of Tfh cells was markedly impaired.

However, the mechanism underlying the IL-21 regulation of Tfh cell polarization has yet to be fully elucidated. A plausible explanation is that IL-21 induces the activation of signal transducer and activator of transcription 3 (STAT3) via IL-21R, promotes expression of the STAT3-induced anti-apoptotic protein B-cell lymphoma-extra large (Bcl-XL), directly restrains Tfh cell apoptosis and enhances its transcriptional activity (30). These conclusions are consistent with previous studies demonstrating that IL-21R is broadly expressed by T cells. The expression of STAT3 and Bcl-XL are increased in UC and CAC (31-33), suggesting that IL-21 activates STAT3 in the tumor initiating cells of WT and IL-21KO mice, but not in epithelial cells. Another possibility is that active STAT3 facilitates the induction of Bcl-6 and promotes the differentiation of Tfh cells from the original lymphocyte.

ICOS is the only homolog of CD28 found in humans and mice. In the absence of ICOS, reduced Tfh cell numbers are observed in mice and humans (34-36). These results suggest that ICOS is significantly important for the development of Tfh cells. Further studies are required to clarify whether ICOS is

necessary for Bcl-6 upregulation and subsequent Bcl-6 controls the Tfh cell differentiation program (37). ICOS signals can also promote the production of IL-21 (37,38). Several studies have suggested that the transcription factor c-Maf, which is downstream of ICOS, regulates the production of IL-21 in developing Tfh cells (39-42). ICOS-deficiency may lead to a defect in c-Maf upregulation, IL-21 production and consequently a defect in the upregulation of IL-21R on Tfh cells. These studies demonstrate that ICOS has a significant effect on the survival of Tfh cells and regulates the IL-21 production of Tfh cells (18,37,43).

In conclusion, the present study assessed the Tfh cell counts and levels of IL-21 to examine whether Tfh cells have an effect during UC. The results confirmed that Tfh cells and IL-21 were upregulated during colitis and indicated that Tfh cells are important in sustaining pathogenicity in UC. The present study also demonstrated that IL-21 promotes the proliferation and secretion of Tfh cells. In addition, more Tfh cells enables the production of more IL-21. Associated cytokines, including ICOS, are able to stimulate Tfh cells, which enhance the production of IL-21 and amplify a positive feedback loop, which assists in stabilizing the Tfh cell line and magnifying inflammation (35-38,43). These results suggested that IL-21 could be a new and potential target for therapeutic agents in UC patients. This was further substantiated by the demonstration that administration of anti-IL-21 following DSS-induced colitis partly attenuated the ongoing inflammation. This may be successful in inhibiting the development of UC when Tfh cell differentiation is restrained in a specific way, however, further investigation is required to demonstrate this.

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